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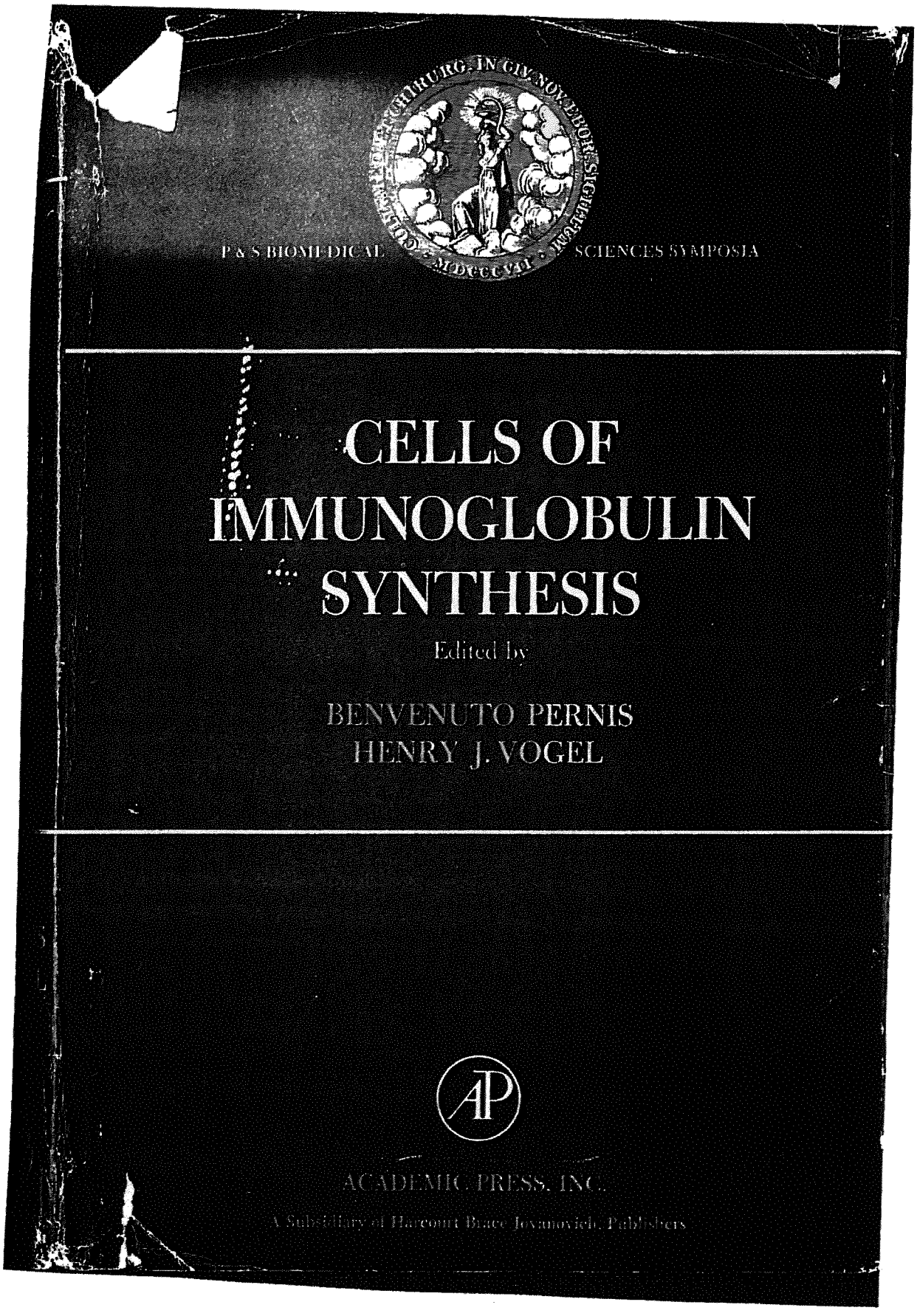
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CELLS OF IMMUNOGLOBULIN SYNTHESIS
Edited by
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Comparative Aspects of *in Vitro* and Cellular Assembly of Immunoglobulins

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The five classes of vertebrate immunoglobulins share a common underlying tetrameric structure, in which two heavy (H) and two light (L) chains are joined together by interchain disulfide bonds and by strong noncovalent interactions (1–4; see also reviews 5–11). Figure 1 is a schematic representation of this general structure with varying numbers and locations of interchain disulfides shown as dashed lines. In most cases, a single disulfide bond links heavy to light chains, and anywhere from one to five disulfides in the hinge region form inter-heavy chain bonds, the actual number depending on heavy chain class and subclass in each species of animal.

The figure also suggests the predominant sites of noncovalent interactions between the domains, drawn as contacting loops, each closed by an intrachain disulfide bond.

The presence of both noncovalent and covalent interactions in a multisubunit structure allows one to consider three distinctive (although not necessarily independent) phases of assembly: (a) folding of the individual chains during and/or after synthesis, which may include closure of the intrachain disulfide bonds; (b) noncovalent association of the folded chains into a tetrameric structure, and (c) covalent assembly through oxidation of the pairs of reduced half-cystine residue that form the correct interchain disulfide bonds. In the synthesis of all classes of immunoglobulins, post-transcriptional modifications such as carbohydrate addition or proteolytic cleavage may occur, and in two of

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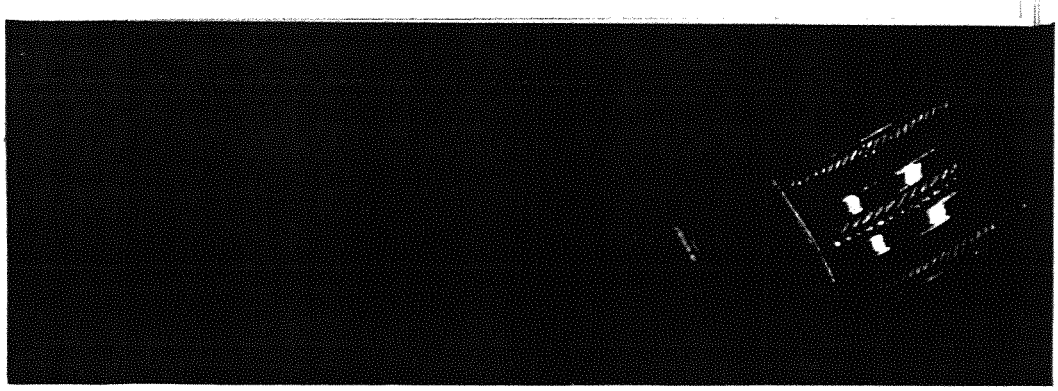


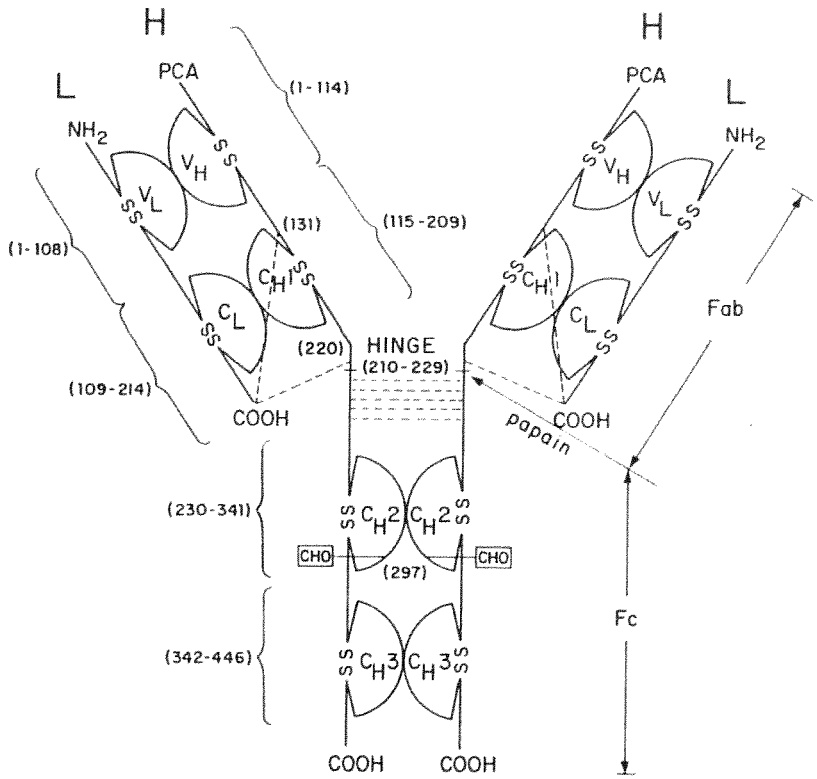


Fig. 1. The structure of immunoglobulin G proteins. The H and L chains are represented by lines showing their N-terminal and C-terminal ends. (PCA is pyrrolidone carboxylic acid, which is the N-terminal residue of most H chains.) The Fab and Fc fragments resulting from papain cleavage are shown on the right. The 12 intrachain disulfides are shown at their relative positions along the chains. The domains of the chains are represented by semicircles which touch at regions of noncovalent interaction. Since the number and location of the interchain disulfides are variable depending on the IgG subclass, only their possible locations are indicated by dashed lines. The residue numbers refer to the human IgG_1 protein (*Eu*) (11a). The sites of carbohydrate attachment to the H chain of *Eu* are indicated by the symbol CHO. Adapted from figures published by Edelman and Gall (5), Dorrington and Tanford (8), and Milstein and Pink (7); taken from Sears (12).

the classes (IgA and IgM), polymerization of the fundamental four-chain unit into larger structures with additional disulfide bonds and peptide components represents a final assembly process (13–16).

Phase (a), that is the rates and pathways of individual domain and chain folding, is under investigation in our laboratory at present, and

will not concern us in this paper except for a few brief remarks at the conclusion. Phases (b) and (c), which describe the self-assembly steps, are the subjects of this talk.

In an early one of the long and important series of papers by Scharff and his associates on intracellular assembly of immunoglobulins, it was noted that assembly of the four-chain structure could occur by three pathways (17):

$$\text{I. } H + L \rightarrow HL \xrightarrow{HL} H_2L_2$$
$$\text{I. } H + H \rightarrow H_2 \xrightarrow{L} H_2L \xrightarrow{L} H_2L_2$$
$$\text{III. } H + L \rightarrow HL \xrightarrow{H} H_2L \xrightarrow{L} H_2L_2$$

Although these pathways can represent either noncovalent or covalent assembly (18, and see below) the experimental procedures in both intracellular and *in vitro* studies more often identify the covalent intermediate states, and we shall therefore first direct our attention to the covalent process. In each of these pathways, at least one of the three possible covalent intermediates—HL, H_2, H_2L—does not occur. Consequently if only one of the covalent routes were used in the assembly of a particular protein, the pathway is established by analysis of intermediates. Studies on many mouse myeloma proteins, using both tumor and cultured cells, on several human meylomas as well as on normal mouse and rabbit lymph node cells (reviewed by Scharff, 18) have revealed that there are preferred pathways of assembly, intracellularly, but that minor pathways invariably occur. In general, the major pathway depends on the structure of the heavy chain, but the relative amounts of the covalent intermediates and the kinetics of assembly vary from tumor to tumor, with average half-times about 7 min for complete covalent assembly of the chains (19,20). By way of summary, mouse IgG_1, IgG_{2a} and IgA assemble predominantly via pathway (II); IgM assembles mainly through (I), and IgG_{2b} assembles through (I) and (III). An especially interesting case is that of the MPC-11 myeloma protein, whose assembly has been studied in both tumor and cultured cells (21). In both instances, HL half-molecules are formed in significant quantities, but in the tumor cells HL intermediates are blocked toward further assembly and secreted as covalent half-molecules. We shall return to this case later.

Our own efforts have been concentrated entirely on developing an *in vitro* system to serve as a counterpart to these cellular investigations (22–28). Two facts, applying equally to the *in vivo* and *in vitro* situations, make these studies possible. The first is that the assembly process is relatively slow, so that the time dependence of the growth and decay of intermediates can be followed. The second is that owing to

the molecular weight difference between heavy and light chains, and to the composition of intermediates, the progress of the reassembly reaction is readily followed by SDS gel analysis. Thus the observable species L, H, HL, H_2, H_2L, and H_2L_2 form a series of increasing molecular weight, the difference being approximately 25,000 daltons between succeeding members.

The protein we have studied most extensively, IgG^{Fro}, is a human $IgG_{1\kappa}$ generously provided over a period of several years by Dr. E. F. Osserman, and isolated from the plasma of a patient with the clinical symptoms of a plasma cell dyscrasia (29). In early experiments (22,23), we sought to establish an analytical approach to the complex reoxidation kinetics and, at the same time, find a basis for comparison with studies of intracellular covalent assembly carried out by other investigators. To do this, we isolated the covalent component of assembly by selective reduction of the interchain disulfides and then studied the reoxidative behavior of the molecule under nondissociating conditions, in which the pre-existing state of noncovalent chain association is maintained even after reduction of the interchain cystine bonds.

Figure 2 shows a representative experiment of this kind. At various times during reoxidation, samples are removed and either alkylated for gel analysis or immediately reacted with Ellmann's reagent (30) for analysis of residual free sulfhydryl titer. Together with the use of methods for the quantitative determination of the concentration of each of the molecular components directly from spectroscopic scans of the gels (23), these experimental procedures enable us to present the kinetics of reoxidation in two different ways, as depicted in Fig. 3. The right side panel simultaneously shows the time-dependent variation in concentration of all observable molecular species and the variation with time of sulfhydryl titer. This is the customary way to display kinetic data and it provides half-times for sulfhydryl disappearance and appearance of the completely reassembled H_2L_2. Moreover, the levels achieved by the intermediates is readily discerned, suggesting in the present case that reoxidation is clearly not restricted to only one of the three pathways (I), (II), or (III). Beyond this information, however, this method of presenting the data is far too complicated for mechanistic analysis. Accordingly, we have chosen to utilize a plot of concentration versus average sulfhydryl titer (left panel of Fig. 3), making time an implicit variable. This method has two important advantages over the conventional function. The first is that it minimizes a major problem inherent in kinetics of air oxidation of thiols, the oxidation kinetics of which are notoriously sensitive to certain trace metals in the buffers, to stirring rates, and other variables that are difficult to

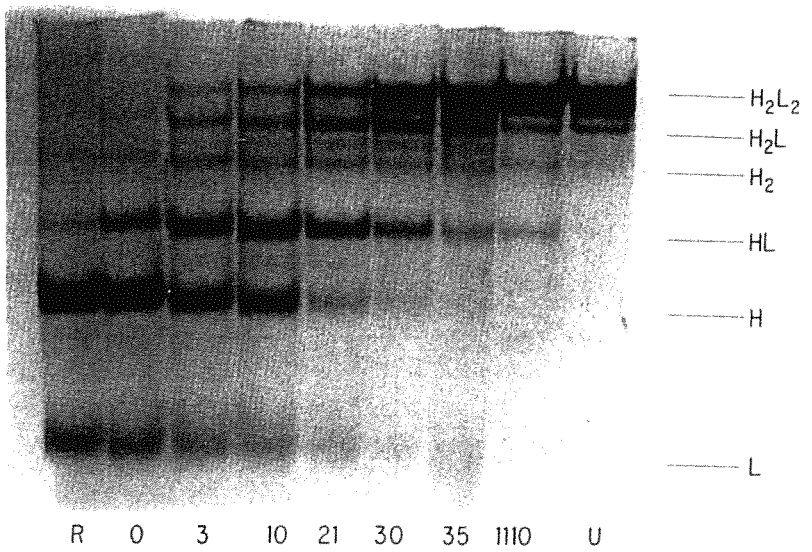


Fig. 2. Strips cut from 5% sodium dodecyl sulfate-polyacrylamide gel slabs stained with Coomassie brilliant blue. Each well (top) was loaded with 24 μg of protein. The various components are identified at the right. The wells labeled U and R represent an unreduced sample and a reduced and alkylated sample taken prior to reoxidation. The remaining strips contain samples alkylated at various times (indicated in minutes at the bottom) in a pH 3.2 → 7.5 reoxidation of reduced *Fro* at a concentration of 4.6 μM. The zero time sample was taken immediately after the protein emerged from the Bio-Gel P2 column. [Taken from Sears *et al.* (22).]

control. The second advantage is that this method makes the experimental results amenable to theoretical analysis of a number of important fundamental questions: What is a random reoxidation and does the system depart from random behavior? Is it possible to discern cooperativity in the covalent assembly, in the sense that the formation of a particular bond alters the rate of formation of another? Is the formation of any one bond favored over any other and, if so, by how much?

Figure 4 shows what random reoxidation would look like for molecules possessing different numbers of inter-heavy chain disulfide bonds. Panel B, for example, corresponds to human IgG_1 and IgG_4; Panel D to mouse IgG_{2b} and so forth. By random reoxidation, we mean that no one bond is favored over any other throughout the course of the covalent assembly. The only restriction is that the half-cystines are correctly paired.

In the upper part of this figure the components are given in molar

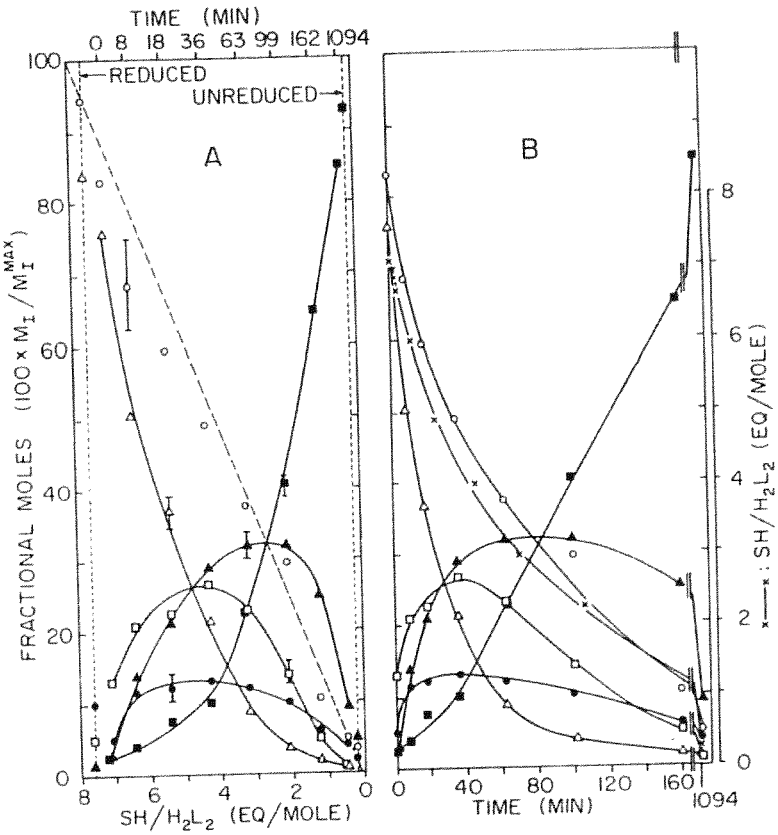


Fig. 3. Analysis of a pH 5.5→7.5 reoxidation (protein concentration, 3.3 μM. [Taken from Sears *et al.* (22).]

quantities, as would be measured in an *in vitro* experiment such as that of Fig. 3. In the lower half, components are given in intensities, such as counts of radioactivity, as might be measured in a cellular pulse-chase experiment. For the latter plots, it is assumed that H and L are synthesized in equimolar ratios. Deviation from the curves in Fig. 4 serves to define a preferred pathway for the disulfide bonding arrangements drawn. Comparison of Fig. 3 with the upper part of Panel B shows that the reoxidation of IgG^{Fro} is not random.

With the analytical experience gained from these "nondissociated" conditions, we proceeded to examine re-assembly after complete dissociation and separation of component heavy and light chains, fol-

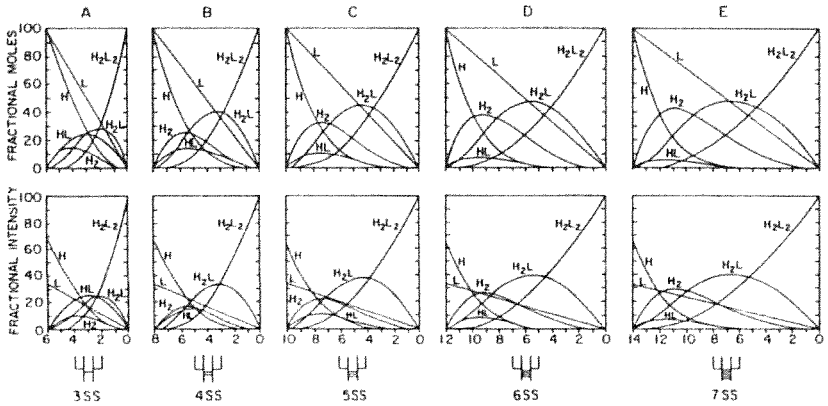


Fig. 4. The hypothetical restricted random reoxidation curves for various patterns of interchain bonding schematically illustrated at the bottom. Each HL and HH bond is assumed to form independently and with the same probability. In the upper panels the components are represented in terms of fractional moles. In the lower panels they are represented in terms of fractional intensities, where intensity here means any conserved property of the H and L chains such as staining intensity or radioactive label. In converting the upper curves to the lower ones, it was assumed that, whatever the property is, it manifests itself in exactly a 2 : 1 ratio for H and L chains. [Taken from Sears *et al.* (22).]

lowed by recombination at various ratios (23,24,31). In particular, it is of interest to know whether actual disruption of the noncovalent bonds leads to any alteration in kinetics and pathway, and whether reoxidation under conditions of light chain excess differs from that at equimolar ratios.

Reoxidation at equimolar levels of H and L chains after chain separation in 1 *M* propionic acid is similar in rate and in assembly pattern to experiments without prior chain separation. Figure 5 shows a reoxidation experiment in which light chains exceed heavy chains in a ratio of greater than 2 to 1. The main additional feature to note is the appearance of a low and almost constant level of covalent L_2 dimer while H_2L_2 is being formed, followed by an increase that begins only after the main assembly reaction is virtually complete. At long times, virtually all of the excess light chain hs been converted into L_2 dimer.

Table I summarizes a number of parameters in the unseparated and separated chain experiments. The main points to be noted are that the average initial rates of sulfhydryl disappearance are the same for both kinds of experiments when the H : L ratio is one, and that the rates of covalent assembly are slowed when L chain is present in excess.

Several other kinds of experiments are possible when partially re-

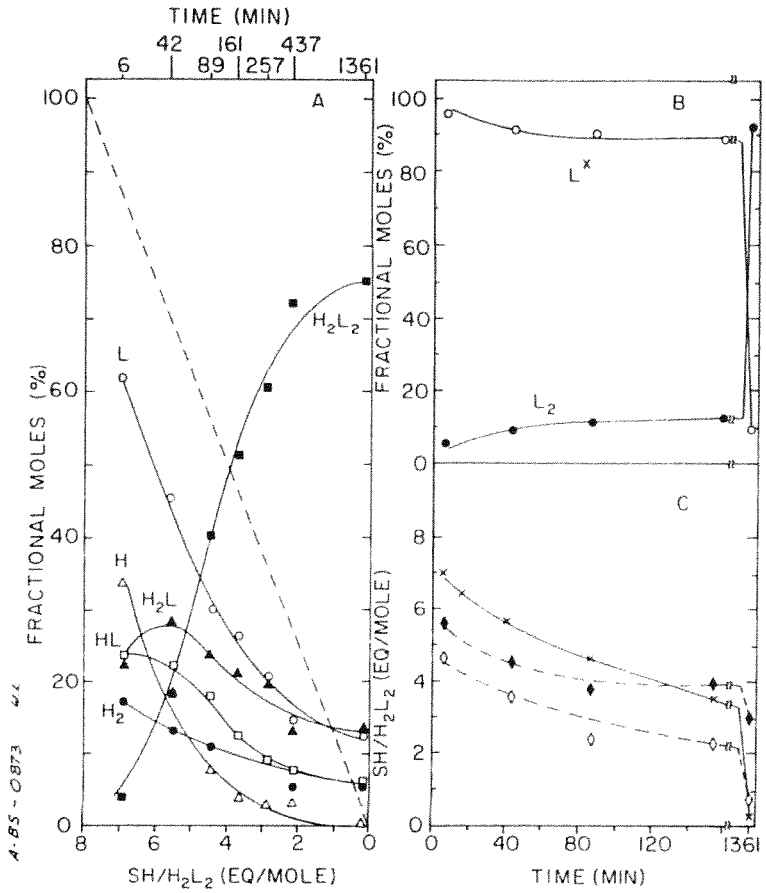


Fig. 5. Recombined chain reoxidation; $R_{L/H} = 2.28$. After reduction of IgG(*Fro*) by DTT, the H and L chains were separated by chromatography on Sephadex G100 in N_2 aerated, 1 *M* propionic acid–1 m*M* EDTA (pH 2.3). The L and H pools were separately chromatographed on Bio-Gel P2 in N_2 aerated 10 m*M* HOAc (pH 3.2), and then mixed in a molar ratio of 2.28:1 just prior to raising the pH and ionic strength for reoxidation. In the final reoxidation mixture $(L)_T = 10.5\ \mu M$, $(H)_T = 4.6\ \mu M$, and $(H_2L_2)_T = 2.3\ \mu M$. (A) The L line and the measured SH titer (abscissa) are corrected here for the L excess over H. (B) Fractional moles of the excess L versus time. On the sodium dodecyl sulfate gels, the excess L appears in two forms: either as a monomer, L (○), or as a covalent dimer, L_2 (●). The ordinate has been normalized relative to the total concentration of excess L chains ($L^x + 2L_2$) which was 0.75 μM in this case. (C)The measured and calculated SH titers versus time as in Fig. 1B. The SH titers are corrected for the SH contribution of L^x and are determined relative to $(H_2L_2)_L$. [Taken from Sears *et al.* (23).]

TABLE I
Comparisons between Unseparated Chain and Recombined Chain Reoxidations[a]

$(H_2L_2)_T$[b]	$R_{L/H}$[c]	$fM_{H_2}max(r)$[d]	$fM_{HL}max(r)$[d]	$fM_{H_2L}max(r)$[d]	t_{av}[e]/SH
		Unseparated chain reoxidations			
3.4	1	25[f]	33(4.8)	28(3.1)	16
3.5	1	18(3.4)	26(4.9)	27(3.4)	18
		Separated-recombined chain reoxidations			
2.9	1.13	24(7.4)	29(6.7)	34(5.0)	15
4.7	1.0	27(6.4)	27(7.1)	43(3.9)	17
2.1	2.19	12[f]	31[f]	26(3.6)	[f]
2.0	2.10	27(7.5)	20(5.9)	33(3.8)	33
1.8	2.28	17(7.0)	24(7.0)	28(5.6)	43
2.2	2.03	26[f]	30(4.2)	22(3.4)	42
1.7	3.46	17(3.9)	30(4.5)	36(3.6)	>50
1.7	3.26	32(7.6)	20(4.9)	39(3.2)	19
1.4	2.80	33(3.9)	25(5.3)	15(3.9)	>50
1.7	3.21	25[f]	16(7.0)	26(4.1)	>50

[a] All experiments are 3.2 → 7.5 reoxidations.
[b] Total possible concentration of H_2L_2 in units of μM.
[c] Molar ratio of L to H.
[d] Fractional moles, in percent; r in parentheses is the corresponding number of SH equivalents per H_2L_2 at the maximum.
[e] Average time in minutes per unit change in the measured SH titer from time zero to the time r = 4.
[f] Insufficient data. [Adapted from Sears *et al.* (23).]

duced, separated H and L chain fractions are prepared. For example, H chain oxidation to H_2 dimers can be studied without the competing and complicating formation of HL bonds (12,23,24). Ordinarily, H chains are not well behaved when alone in the reoxidizing buffer. In addition to the expected formation of the H_2 dimers, higher molecular weight covalent aggregates also readily form, as anticipated from early studies on the behavior of alkylated γ chains in acid and at neutral pH (32,33). In such experiments, the formation of aggregates is too rapid to allow any conclusion about the reoxidation kinetics of inter-heavy chain disulfide bond formation in the absence of L chains.

Three kinds of experiments using partially reduced H chains in combination with modified L chain species are shown in Figs. 6–8. In Fig 6, H chains are oxidized to H_2 dimers in the presence of alkylated light chains, which serve to solubilize the H chains but are blocked from forming HL bonds (12,23). In Fig. 7, solubilization is effected by

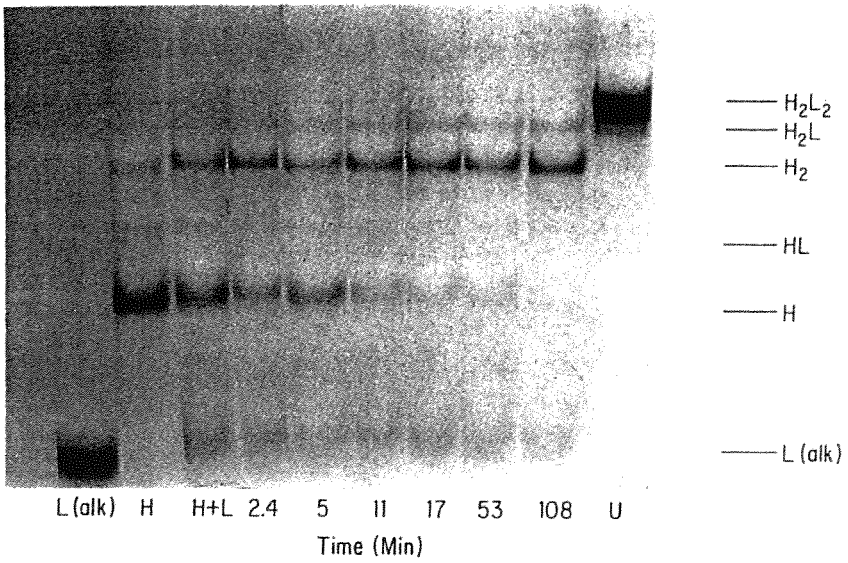


Fig. 6. Sodium dodecyl sulfate-polyacrylamide gels of the reoxidation of H chains mixed with prealkylated L chains, L(alk); $R_{L/H} = 1.5$. The samples in the gels designated L(alk) and H were taken from the respective pools just prior to their being mixed for reoxidation. The gel designated by U is that of an unreduced sample of $IgG^{(Fro)}$, included for reference. In the final reoxidation mixture $(L)_T = 5.5\ \mu M$, $(H)_T = 3.7\ \mu M$, and $(H_2L_2)_T = 1.85\ \mu M$. [Taken from Sears *et al.* (23).]

utilizing V_L fragments [from a 37°C pepsin digestion of Bence-Jones (*Fro*) protein (31)]. V_L does not inhibit covalent polymerization of H chain as well as whole, alkylated L chain, but the reaction is sufficiently orderly that the kinetics of H_2 formation can be studied in the absence of the C_L–C_{H1} domain interaction.

Finally, Fig. 8 shows the reoxidation of H chains in the presence of the L_2 covalent dimer (27). This is a striking experiment which shows that L_2 is rapidly incorporated into the normal pathway of covalent assembly through either of two interchange reactions:

$$\text{IV.} \quad H + L_2 \rightarrow HL + L$$
$$\text{IV}'. \quad H_2 + L_2 \rightarrow H_2L + L$$

Thus, when excess L chain is present, an additional pathway for assembly is provided. Any excess L that has been converted into L_2 may react with either H or H_2 through a disulfide interchange reaction.

To summarize thus far, the overall reoxidative assembly patterns in experiments with H and L separated prior to recombination are similar

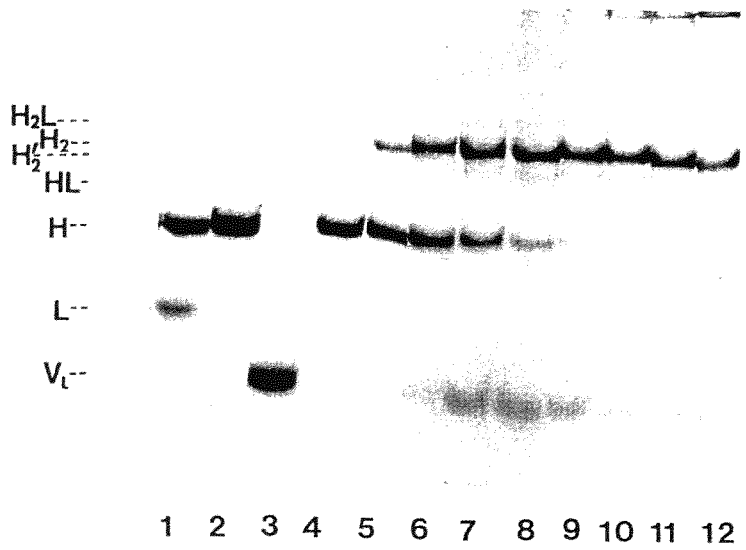


Fig. 7. SDS polyacrylamide gels of $H_{SH} + V_L$ reoxidation. Wells 1 and 2 represent the partially-reduced myeloma protein and the purified H chain fraction, respectively. Well 3 is the V_L fragment isolated from a 37°C partial pepsin digest of B J protein. Well 4 is the $H + V_L$ mixture (1 : 1) that was alkylated before raising the pH. Wells 5–12 represent aliquots from the reoxidation mixture alkylated at various times. Well 5: 3.5 min; well 6: 18.0 min; well 7: 39.5 min; well 8: 73.0 min; well 9: 102.0 min; well 10: 145.5 min; well 11: 192.0 min; well 12: 1080.0 min. [Taken from Kazin (31).]

to those observed when the chains remain noncovalently associated throughout. With equimolar mixtures of H and L, the reoxidation rates also are similar to those of unseparated chains. However, when L chains are present in excess, the overall *in vitro* rates of covalent assembly are generally diminished, probably indicating transient nonproductive interactions. At the highest molar excesses of L (3 : 1), the assembly pahtways may also be modified. In all experiments with excess L chains, covalent L_2 dimers form at rates which are comparatively slow relative to the H_2L_2 assembly rates. Three kinds of reoxidation experiments with modified L chains are also described. In the first, the free half-cystine of L is irreversibly blocked by reaction with iodoacetamide, and the alkylated L chains are recombined with reduced H chains. This experiment isolates the reactions in which H_2 disulfides are formed without the accompanying formation of HL bonds. Although the alkylated L chains do not play a direct role in the reoxidation, their presence is required to inhibit aggregation and pre-

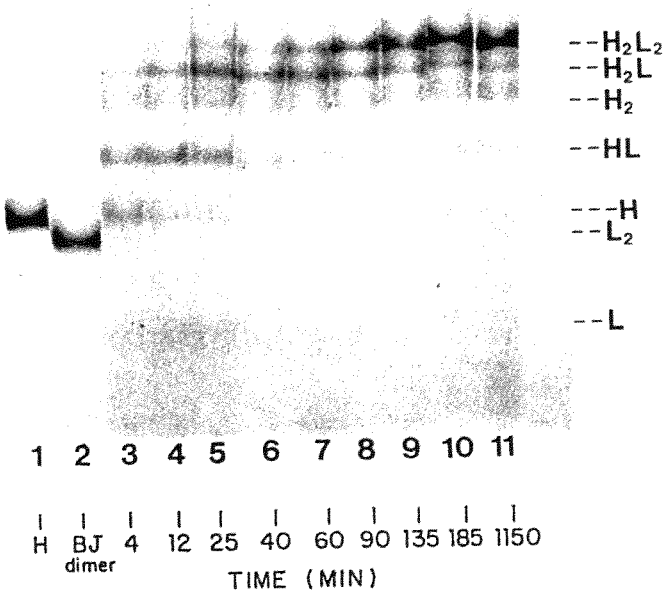


Fig. 8. The reoxidation of partially reduced heavy (H) chain with light chain covalent dimer (L_2) shown on SDS polyacrylamide gel. Wells 1 and 2 represent the purified H chain and Bence-Jones (BJ) covalent L chain dimer, respectively, after Bio-Gel P2 chromatography. The H and L_2 fractions were combined under N_2 at an equimolar ratio in 10 m*M* acetic acid (final ratio of total L chain of H chain, 1 : 36). The pH of the mixture was then raised to 7.5 and the mixture was exposed to air. The final concentration of H_2L_2 was 1.6 μM. Wells 3–11 represent portions from the reoxidation mixture that were immediately alkylated with 1.0 *M* iodoacetamide at the times indicated. All gel samples contained 1% SDS and iodoacetamide. [Taken from Kazin and Beychok (27).]

cipitation of high molecular weight products which otherwise ensue; this suggests a possible biological role for excess L *in vivo*. In the second kind of experiment, H chain is oxidized in the presence of the isolated V_L domain. In the third kind of experiment, covalent L_2 dimers are mixed with reduced H chains. L_2 rapidly disappears with the concurrent appearance of HL, H_2L, and fully assembled H_2L_2. H_2 dimers are also reactive in this process.

With respect to the question of random versus nonrandom behavior in the covalent assembly process, Sears and Beychok (24) have carried out a theoretical analysis in which the experimental results are exam-

ined in terms of the relative probabilities of H–H and H–L bond formation throughout the course of the reoxidation experiment.

Figure 9 compares the observed probabilities of formation of HH and HL bonds, and their ratio, during an unseparated chain reoxidation, compared to expected theoretical random probabilities. At the outset of the reaction, all bonds can form, but the probability that an HL bond is formed initially is about twice as great as that of an HH

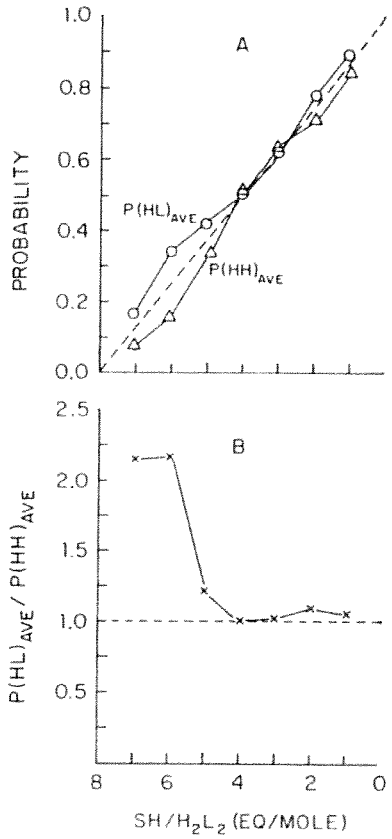


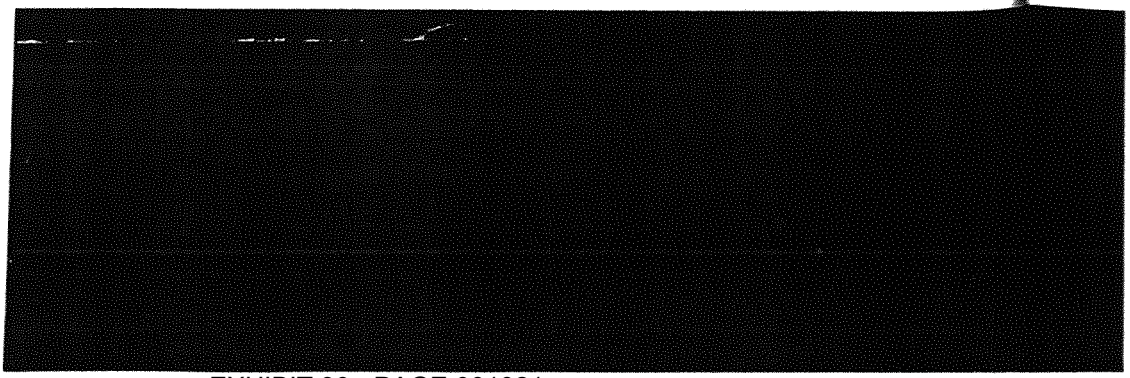
Fig. 9. Disulfide bond formation probabilities for an unseparated chain, 3.2 → 7.5 reoxidation. (A) The average probabilities for forming either an inter-HL bond (○——○) or an inter-HH bond (△——△) were determined from the experimental reoxidation profiles for L, H, etc. and the corresponding SH titers. The diagonal dashed line is the corresponding probability expected for both of these bonds if the reoxidation were random. (B) The ratio (×——×) of the average probabilities shown in (A). The horizontal dashed lines again refer to the random case. [Taken from Sears and Beychok (24).]

bond. As the reaction proceeds, this preference diminishes until about midway in covalent bond formation when the two probabilities become equal.

The essential features of the foregoing studies, then, are: (1) The rate and pattern of covalent chain assembly are qualitatively the same whether the reduced H and L chains remain associated or are separated by propionic acid and recombined prior to establishing reoxidation conditions. (2) Molar excess of L over H chains—up to threefold—do not have a marked effect on the pattern of covalent H_2L_2 assembly, although at molar ratios of greater than 3 : 1, the values of fM_{H2}^{max} are increased (in three of four experiments) and the overall rates of sulfhydryl disappearance are diminished, suggesting the possible occurrence of nonproductive transient intermediates. (3) The basic pattern of covalent assembly appears to be characteristic of human IgG_1 proteins in general since Petersen and Dorrington (34) and, very recently, Kishida *et al.* (35) found qualitatively similar reoxidation patterns in studies of other myeloma proteins. (4) Reduced H chains readily reoxidize to H_2 dimers in the presence of alkylated L chains, which have irreversibly lost their capacity to form inter-HL disulfide bonds. (5) A disulfide bond between L chains in the form of L_2 dimers does not prevent the formation of inter-HL disulfide bonds; on the contrary, mixtures of reduced H chains and covalent L_2 dimers readily reoxidize to fully assembled H_2L_2 molecules with concurrent disappearance of L_2 through disulfide exchange.

From the first two observations above, one can draw the broad conclusion that the covalent chain assembly of IgG^{Fro} *in vitro* proceeds in a characteristic fashion which, under the conditions of these experiments, at least, is qualitatively independent of whether the chains are physically separated prior to reoxidation and whether L exceeds H in concentration. The basic similarity between the unseparated chain and recombined chain reoxidations is probably accounted for by the very strong, essentially irreversible, noncovalent affinities between chains which maintain H and L in the assembled H_2L_2 form in both types of experiment. This is the essential concept underlying our analysis of the *in vitro* assembly of IgG molecules.

To prove that the *in vitro* reoxidation conditions establish a stable noncovalently associated tetramer structure very rapidly, we undertook a series of stopped-flow experiments on the kinetics of noncovalent interaction of heavy and light chains in IgG^{Fro} (28). A fluorescent probe, N-(iodoacetylaminoethyl)-8-naphthylamine-1-sulfonic acid (1,8-I-AEDANS) reacts stoichiometrically with the COOH-terminal cysteine residue of the partially reduced L^{Fro} chain. The fluorescence



spectra of the modified chain and of the mixture of this chain with an equimolar amount of H chain are shown in Fig. 10, and Fig. 11 shows the fluorimetric titration of L-AEDANS with H chain. The rate of association of these chains is displayed as a second order plot in Fig. 12. The rate constant for this reaction is 6×10^6 liters $mol^{-1}sec^{-1}$ at 20°C, probe, N-(iodoacetylaminoethyl)-8-naphthlamine-1-sulfonic acid (1,8-I-AEDANS) reacts stoichiometrically with the COOH-terminal (36–40).

Since the reaction is second order, the half time is inversely proportional to initial reactant concentration. For the case shown, with a light

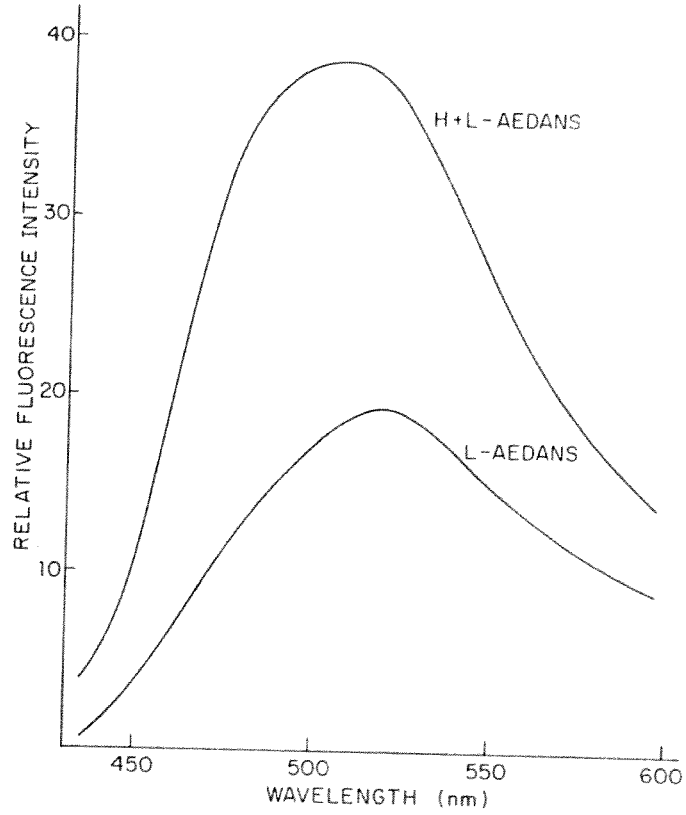


Fig. 10. Emission spectra of L-AEDANS and H + L-AEDANS. The L-AEDANS concentration was 0.20 μM in TE buffer. A small volume of H chain solution was added to a final concentration of 0.22 μM. Temperature was 20°C. Excitation λ, 343 nm; band width was 5 nm for both excitation and emission. [Taken from Friedman *et al.* (28).]

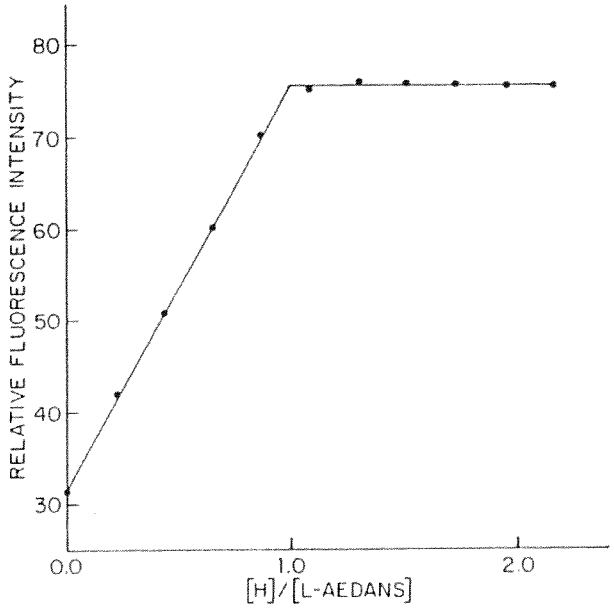


Fig. 11. Fluorometric titration of L-AEDANS with H chain. Small volumes of 2.8 μM H chain were added to a 0.13 μM solution of L-AEDANS in TE buffer and the fluorescent intensities determined after equilibration. Temperature was 20°C. Excitation λ, 343 nm, band width 5 nm; emission λ, 488 nm, band width 10 nm. [Taken from Friedman *et al.* (28).]

chain concentration of $3.3 \times 10^{-8}\,M$ and heavy chain $5.4 \times 10^{-8}\,M$, the half time is 2.5 sec, which may be compared with the average of about 15 min for covalent bond formation at still higher concentrations.

We turn now to a comparison of the *in vitro* and cellular assembly. To begin with, it is important to note that the main qualitative results of our *in vitro* work with IgG^{Fro} are not special in kind, or specific for a single protein. As noted above, Petersen and Dorrington (34) and Kishida *et al.* (35) working with different proteins find qualitatively similar patterns of *in vitro* assembly, although their methods of analysis differ considerably from ours. Furthermore other investigations, including work with mouse proteins (41,42), all suggest that there is a fundamental similarity between *in vitro* and cellular processes with respect to the dominant influence of H chain structure on the profile of assembly intermediates (pathway of assembly), as was suggested in the very earliest *in vivo* studies of Scharff's group. This establishes a

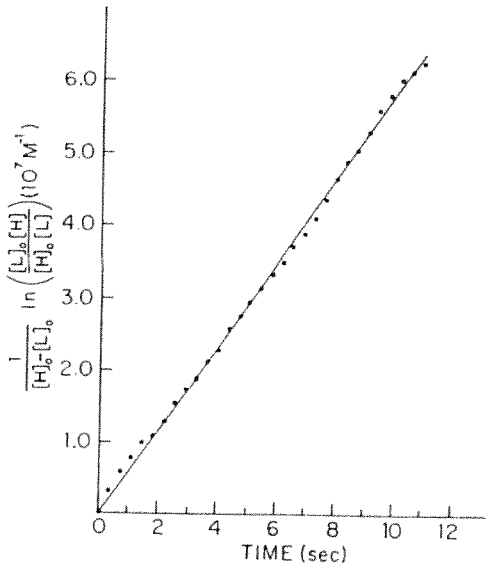


Fig. 12. Second order plot of the association of H with L-AEDANS. Final concentrations were 33 nm L-AEDANS and 54 nm H chain in 25 m*M* Tris-HCl, 21 m*M* NaCl, 1 m*M* sodium acetate, 0.5 m*M* EDTA, pH 7.5. Temperature, 20°C. [Taken from Friedman *et al.* (28).]

structural basis for control of assembly that is common to cellular and *in vitro* studies and is largely uninfluenced by the specific environmental features of the intracellular assembly sites.

Second, there is at least a rough correspondence, which must have a structural basis, in terms of the rates of assembly. For example, the mouse protein MOPC-31C assembles very slowly intracellularly with less than 40% completion in 18 min compared to an average of about 70% completion (18). Percy *et al.* (41,42) and our laboratory (31) have examined the *in vitro* assembly. We have found low levels of completion (<40% at greater than 100 hr), and a half-time for sulfhydryl disappearance of more than 200 min.

Third, there may be related effects caused by L chain excess in both kinds of experiments. *In vitro*, excess L chain experiments have certain unique features (31): (1) a diminished rate of sulfhydryl disappearance, (2) an inhibition of the usual H chain aggregation and precipitation observed at later times in other reoxidations, (3) a decreased rate of L chain dimerization compared to L chains in solution alone.

Obviously, the L chains are not free to interact because they are complexed with H chains. L chain dimerization does not take place until the H chain sulfhydryls have oxidized. Formation of transient nonproductive intermediates such as HL_2 could account for these effects.

As noted above, the assembly of H and L chains differs in MPC-11 tumors and cultured cell lines, although there appear to be no structural differences in the chains. However, the tumor cells synthesize 3.6 to 4-fold excess of L chains, whereas the cells in culture synthesize about 1.7-fold excess L chain. In the case of the tumor cells, HL is made but does not assemble further into H_2L_2. Baumal and Scharff (20) found these half-molecules in noncovalent association with L chains, and suggested the occurrence of nonproductive complexes such as (LH)L(HL). This may be similar to the situation in our excess L chain experiments, where the diminished rate of assembly has been attributed to transient nonproductive complexes, such as HL_2.

These qualitative similarities certainly indicate that cellular self-assembly requires no special mechanism. However, in detail the *in vitro* and *in vivo* assembly processes may differ considerably. For example, the *in vivo* rates are faster where comparable proteins have been studied. Moreover, the instances of assembly blocks and of high levels of kinetic cooperativity are much more securely established in the cellular studies than in the *in vitro* studies reported to date, with the possible exception of the block in a human IgG_4 reported by Petersen and Dorrington (34).

The final difference to be noted is by far the most serious. In the *in vitro* assembly studies, noncovalent association prior to disulfide bond formation is established beyond reasonable doubt. Indeed, the experiment with L_2 covalent dimer (Fig. 8) demonstrates the thermodynamic control over the assembly process exercised by the correct positioning of L and H chains through noncovalent domain associations. In this view, the kinetic cooperativity observed in covalent assembly of IgG^{Fre} represents a fine-tuning of the tetramer structure initially established by rapid noncovalent interactions. In contrast, few if any H and L chains appear to be noncovalently assembled for any measurable length of time prior to interchain disulfide bond formation intracellularly (43; M. D. Scharff, personal communication). If this is not one of the most important differences thus far observed between *in vitro* and *in vivo* assembly, it is surely one of the most interesting and puzzling, and provides an excellent avenue for future investigations of immunoglobulin assembly.

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